Histone H3 Lys79 Methylation is Required for Efficient Nucleotide Excision Repair in a Silenced Locus of *Saccharomyces cerevisiae*

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Supplemental Table 1: Relative expression[1] of certain NER genes between H3 K4,79R mutant and *wt* cells

S.No	Name of Gene	*Fold change*[2]
1	*Rad2*	*NC*[3]
2	*Rad4*	*NC*
3	*Rad14*	*NC*
4	*Rad1*	*NC*
5	*Rad7*	*NC*
6	*Rad10*	*NC*
7	*Rad25*	*NC*
8	*TFB2*	*NC*
9	*TFB3*	*NC*
10	*TFB1*	*NC*
11	*SSL1*	*NC*
12	*Rad3*	*NC*
13	*Rad26*	*NC*
14	*Rad16*	*NC*
15	*Rad23*	*NC*

[1] Data in Table 1 were generated from Microarray data accession number GSE6319 (http://www.ncbi.nlm.nih.gov/geo/) (17)
[2] Fold change was determined as described in Martin et al, 2004 (25).
[3] NC, no change.

SUPLIMENTARY FIGURE LIGANDS

Figure S1: Expression of *HMLα1, RPB2* and *GAL10* genes in H3 K79R and H3 K4,79R mutants. (A) RT-PCR analysis was performed on total RNA from *wt*, H3 K4,79R and H3 K79R cells using primers specific for *RPB2, HMLα1* and *GAL10* gene. The mRNA levels of the *ACT1* gene were used as a loading control. (B) Expression of *HMLα1* in *wt*/Δsir2 and H3 K4,79R/Δ sir2 cells.

Figure S2. MNase digestion patterns for *GAL10* loci in H3 methylation mutants. (A) Spheroplasts were isolated from WY139 (*wt*) and methylation mutants, treated with different concentrations of MNase (10 U/μl stock solution) and genomic DNA hybridized with a probe specific for the *GAL10* ORF. (B) Quantitative analysis of MNase accessibility at *GAL10* is expressed as the ratio of mono- to tri-nucleosome signal at different concentrations of MNase.

Figure S3. NER and MNase accessibility of the *RPB2* locus in Sir2 deletion mutants. (A Western blot showing Sir2 levels in *wt* and H3,K4,79R cells. The level of α-tubulin subunit served as a loading control (lower panel). (B) MNase digestion of WY139 (*wt*) and H3K4,79R mutants. Spheroplasts were isolated, treated with different concentrations of MNase (10 U/μl stock solution) and genomic DNA was isolated, separated by agarose gel electrophoresis, blotted and hybridized with a probe specific for the *RPB2* ORF.

Figure S4. Nucleotide excision repair at the *HML* locus in dot1Δ and H3 K79G mutants. Cells were irradiated with 100 J/m^2 UV light and allowed to repair in the dark at 30^{o}C for various times. The time course of CPD removal from the *HML* locus is shown for the H3 K79G mutants (panel A) and the dot1Δ mutant (panels C). Data represent the mean ±1 SD for three independent experiments. To the right of each graph is shown

MNase digestion profiles for *HML* chromatin from the H3 K4,79R mutant (B) and dot1Δ mutant (D). Data were obtained as described in legend to Figure 3.

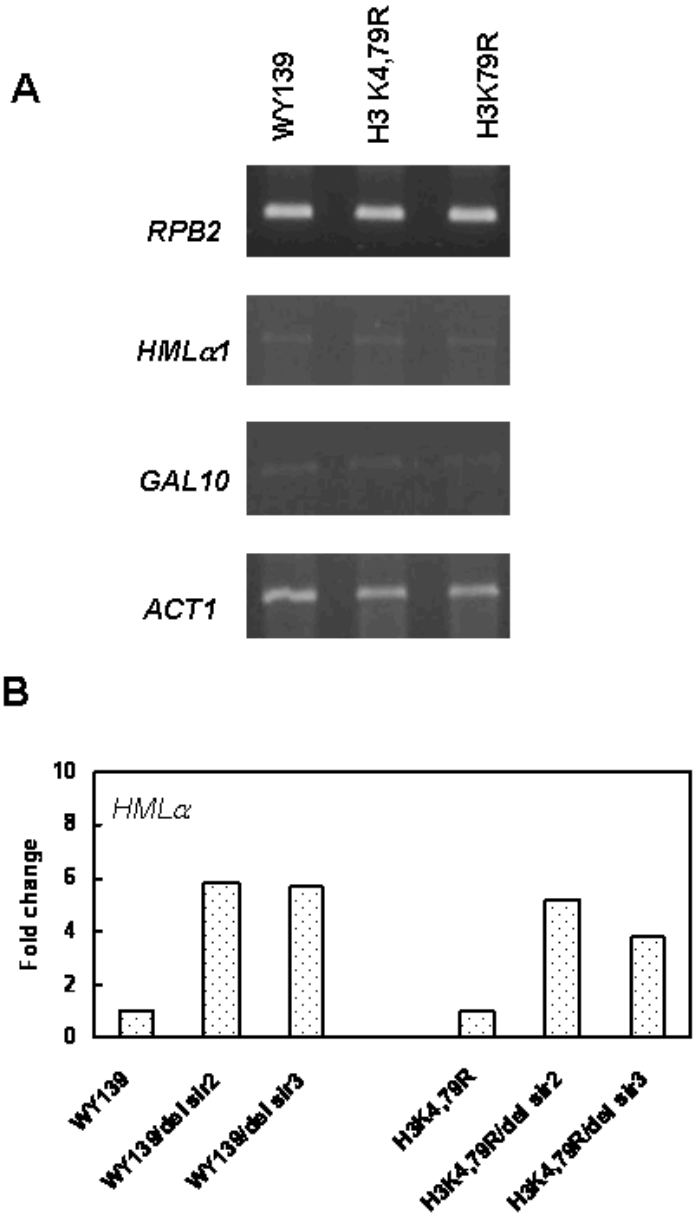

A
WY139
H3 K4,79R
H3K79R
RPB2
HMLα1
GAL10
ACT1
B
HMLα
Fold change
10
8
6
4
2
0
WY139
WY139/del sir2
WY139/del sir3
H3K4,79R
H3K4,79R/del sir2
H3K4,79R/del sir3

Fig S1

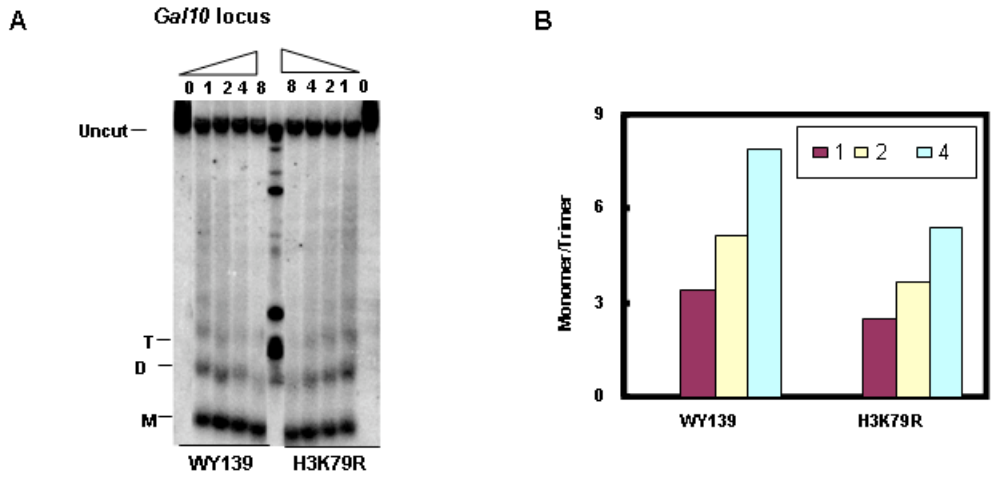
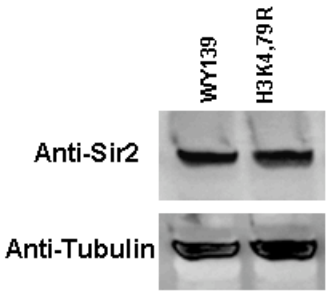

A
Gal10 locus
0 1 2 4 8
8 4 2 1 0
Uncut
T
D
M
WY139
H3K79R
B
Monomer/Trimer
9
6
3
0
1
2
4
WY139
H3K79R

Fig S2

A


WY139
H3K4,79R
Anti-Sir2
Anti-Tubulin

B

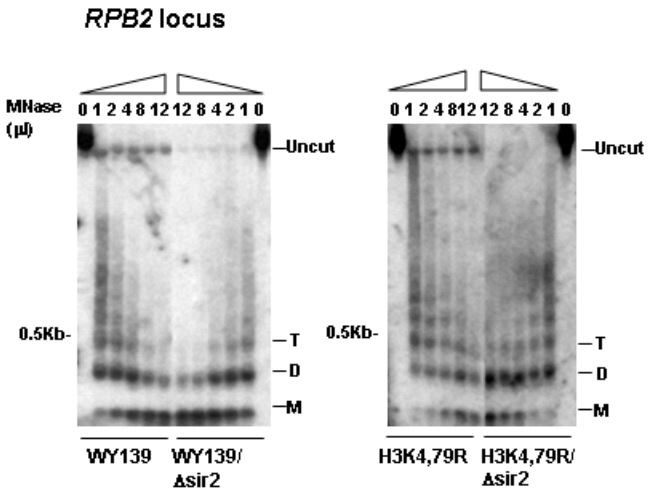

RPB2 locus
MNase (μl)
0 1 2 4 8 12 12 8 4 2 1 0
Uncut
0.5Kb-
T
D
M
WY139
WY139/ Δsir2
H3K4,79R
H3K4,79R/ Δsir2

Fig S3

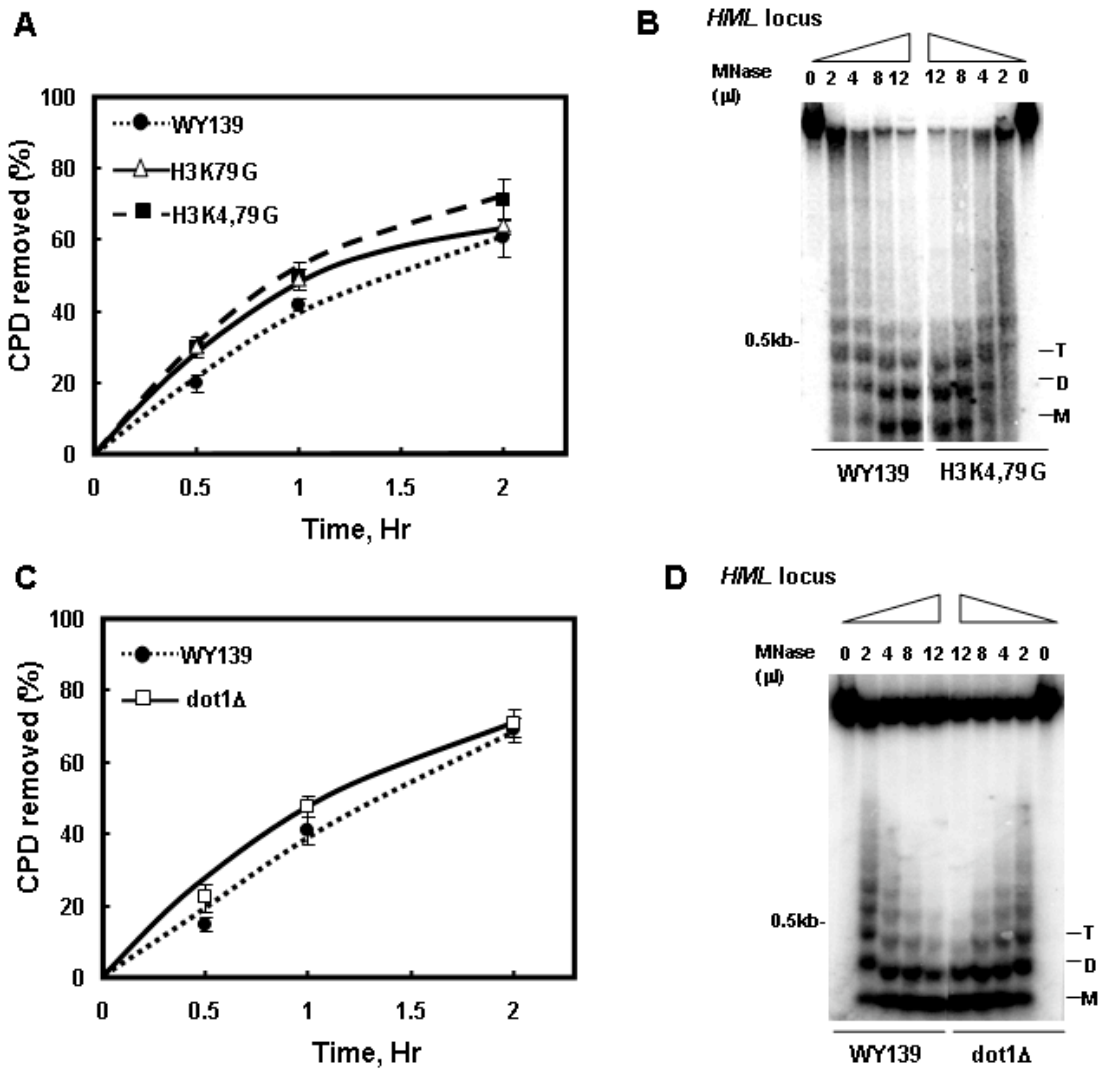

A
CPD removed (%)
WY139
H3K79G
H3K4,79G
Time, Hr
B
HML locus
MNase (μl)
0 2 4 8 12 12 8 4 2 0
0.5kb-
T
D
M
WY139
H3K4,79G
C
CPD removed (%)
WY139
dot1Δ
Time, Hr
D
HML locus
MNase (μl)
0 2 4 8 12 12 8 4 2 0
0.5kb-
T
D
M
WY139
dot1Δ

Fig S4